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TOBACCO INDUSTRY RESEARCH COMMITTEE  
150 East Forty Second Street  
New York 17, N.Y.

#265M  
(M - modified)  
(Cf. #265  
considered on 3/11/60)

Application for Research Grant

Date: April 22, 1960

1. Name of Investigator: Gustave A. Laurenzi, M.D.
2. Title: Assistant Professor of Medicine  
Coordinator of the Division of Respiratory Diseases
3. Institution & Address: Seton Hall College of Medicine  
Medical Center  
Jersey City 4, N.J.
4. Project or Subject: Studies in bronchitis: A correlated investigation of:
  - (a) the effect of smoking on the bacteriology of the respiratory tract of humans, and
  - (b) the effect of cigarette smoke and sulfur dioxide (air pollutant) on the clearing of bacteria from the respiratory tracts of small animals (mice, rats).

5. Detailed Plan of Procedure:

I. Human study: This phase is designed to study the bacteriology of the upper and lower respiratory tract in normals (smokers and non-smokers) and chronic bronchitics (smokers and non-smokers). The bacterial flora of expectorated sputum, oropharyngeal secretions, tracheal secretions, and secretions from the lower bronchial tree will be examined in each subject, and comparisons will be made between the different groups. Tracheal and bronchial specimens in the form of swabs and aspirates will be obtained under sterile conditions by bronchoscopy and by intubation with rubber catheters (as in bronchography). All specimens will be homogenized and plated out on appropriate media. Quantitative bacterial counts by the pour plate method will be done on the aspirates. (Please see section II on attached sheet).

II. Animal Study: Different groups of animals will be exposed to cigarette smoke and sulfur dioxide in a chamber for variable lengths of time. These times will range from acute exposure periods to periods similar to those in which changes in bronchial epithelium have been observed (C. Leuchtenberger, et al). Following these periods the animals will be exposed to a challenging dose of aerosolized bacterial (staphylococci, pneumococci) in a specially designed aerosol system.\* The bacterial aerosol is generated in an air stream at one end of the apparatus, carried past the experimental animals, and then put through a filtering apparatus. This system offers a safe method for the administration of predictable doses of bacteria. At specific time intervals after exposure, groups of animals will be sacrificed; the lungs will be removed aseptically and homogenized; and bacterial counts will be done on the homogenates by

\* please see section III on attached sheet

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counting colonies on agar pour plates. In this way it is possible to determine the number of bacteria cleared in a particular period of time under these special conditions. By similar experiments this past year we have constructed "time-concentration curves",\*\* and the results show that bacteria are cleared from the lungs of normal mice and rats in an exponential manner. Ninety-five to 98 per cent of the bacteria are gone 6 hours after exposure. Time-concentration curves will be determined and compared in the animals exposed to cigarette smoke and those exposed to sulfur dioxide; these will also be compared with the control data cited above. In this way it may be possible to show the effects of these materials on the clearing of bacteria from the animals' respiratory tracts.

6. Budget Plan:

Salaries	\$3,700.00
Expendable Supplies	2,193.00
Permanent Equipment	7,455.00
Overhead (15% of 1 and 2)	884.00
Other	-----

Total \$ 14,232.00

7. Anticipated Duration of Work: This study is planned for one year. The versatility of the permanent equipment, however, makes it possible in the future to apply the aerosol method to many other aspects of the problem.

8. Facilities and Staff Available: There will be adequate office and laboratory space at the medical school. There is ample area for the housing and care of laboratory animals, and culture media and basic bacteriological needs will be supplied by the hospital microbiology department. This project will require the part-time participation of a thoracic surgeon, and a bacteriology technician will also be included. It is planned that a senior medical resident will participate in the study.

9. Additional Requirements: The use of aerosolized cultures of bacteria requires maximum protection for personnel. Concerning this matter I have visited and consulted the Bacterial Warfare Center at Fort Detrick, Maryland, and the School of Public Health and Department of Industrial Hygiene at Harvard. The plan of this experiment and the protective measures we will use meet with their approval.

10. Additional Information (Including relation of work to other projects and other sources of supply): My research activities at Seton Hall will be concerned with chronic bronchitis. My major research project in the department of medicine will be the one proposed here. Along with this study I will conduct a bronchitis clinic at Seton Hall where we will continue to make observations on the natural history, living habits, and management of patients with chronic bronchitis. We hope to incorporate this information into a correlated prevalence study of bronchitis and air pollution in Hudson County, New Jersey. This will be a coordinated effort between the departments of medicine and preventive medicine. Dr. Benedict Duffy, Professor of Preventive Medicine, will head the epidemiological aspects of this investigation.

/s/ Gustave A. Laurenzi, M.D., Dir., of Project  
/s/ Hugh G. Grady, Business Officer

\*\* plots of time after exposure against the mean number of bacteria in the animals' lungs.

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Gustave A. Laurenzi, M.D.

11. (Dr. Hockett has suggested that I include a section on past work, techniques developed, and general objectives).

The correlated study outlined here is a direct extension of the investigations which I have carried out this past year. The examination of expectorated sputum for bacteria is misleading in that secretions from the lower respiratory tract are contaminated by oropharyngeal secretions during the process of expectoration. To get a clearer picture of the bacteriology of the bronchial tree I have obtained secretions from the lower respiratory tract by bronchoscopy. These specimens show that in the normals (including many heavy cigarette smokers), the bronchial tree below the level of the carina is sterile; but in the bronchitics, oropharyngeal organisms\* and pathogens are found consistently in this region. These findings suggest that any effect that cigarette smoke might have on the bronchial tree does not interfere with the normal clearing of bacteria. (This material is being prepared for a preliminary report in the New England Journal of Medicine.)

In our laboratory this year we have constructed an aerosol exposure system for studying the infectivity of microorganisms suspended in air as particles. Many experiments have been done to calibrate the instrument so that with a known aerosol inoculum the number of bacteria in each animal's lungs after a fixed exposure time is predictable. We have worked with a dose by which 80,000 bacteria are deposited in each animal's lungs after thirty minutes of exposure. As noted above, timed killings after exposure have shown that 95 to 98 per cent of all the bacteria are gone in 6 hours. These studies demonstrate the remarkable capacity of the respiratory tract to clear itself of bacteria.

Chronic bronchitis is a major medical, social, and economic problem; yet our knowledge of the incidence and pathogenesis of this condition is incomplete, and methods for its control are seriously inadequate. Although the etiology of chronic bronchitis remains obscure a good deal is known about the factors which hasten a bronchitic's deterioration. By following the course of the bronchitic an important correlation has been made between the increasing disability of the bronchitic and the number of exacerbations of acute bronchitis he suffers. During exacerbations bacterial pathogens are found in the sputum in 50 to 80 per cent of the cases. From this data it appears that infection plays a major role in determining the progress of the bronchitic's deterioration: with repeated infections more and more lung tissue is damaged and pulmonary reserve is compromised. Studies are needed to show what factors upset the normal mechanisms whereby the respiratory tract is able to rid itself of bacteria. How are the bacteria able to take hold, colonize, and cause bronchial infection? Air pollution and smoking have been implicated as contributing factors. After observing bronchitics at three large medical centers the role of these factors seems more involved and unclear to me. I anticipate that the correlated human and animal study proposed here will give objective information fundamental to the entire problem. Of course this information must be tempered with the realization that these isolated effects may not be the only responsible factors.

\* neisseria, non-hemolytic and green streptococci.

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